Dilute Solution Behavior of Dendrimers and Polysaccharides: SEC, ESI-MS, and Computer Modeling

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Dendrimers, the most highly branched structures achievable, have found numerous uses in the chemical, biological, and pharmaceutical fields. We have employed size exclusion chromatography (SEC) with universal calibration to determine molecular weight averages, distributions, intrinsic viscosities, and structural parameters of Starburst dendrimers, dextrans, and the starch degradation polysaccharides known as maltodextrins. Comparisons have been made in the dilute solution behavior of dendrimers and polysaccharides with equivalent weight-average molecular weights. Intrinsic viscosities decreased in the order $[\eta]_{dextran} > [\eta]_{dextrin} > [\eta]_{dendrimer}$. While the difference between dendrimer and polysaccharides may be attributed to the higher branching of the former, which leads to a higher chain density in solution, the difference between dextran and dextrin is likely a result of the variation in solution behavior of α-(1→6) vs α-(1→4) linked carbohydrates. The solution behavior of the maltodextrins studied indicates that debranching in their manufacture appears to have been more thorough than in that of β-limiting dextrins studied by other groups. Comparison of molecular radii obtained from SEC data to radii from molecular dynamics studies show Starburst dendrimers behave as θ-stars with functionality between 1 and 4. Additionally, electrospray ionization mass spectrometry was employed to determine M_w, M_n, and PD of Astramol dendrimers.

Dendrimers, which possess a branch point at every monomer unit, are potentially the most highly branched structures that may exist.[1] Since their discovery nearly twenty years ago,[2−4] this unusual class of polymers has found an array of scientific, engineering, and clinical applications. They may serve as drug delivery carriers,[5] chromatographic support media,[6] DNA transport structures,[7] NMR contrast agents,[8] and molecular wires,[5] as well as serving in the field of supramolecular chemistry with roles ranging from molecular recognition to self-assembly.[9] Differences in the swelling behavior of dendrimers in various solvents allow making use of their internal cavities for carrying molecular "baggage" which may be released when the dendrimer finds itself in a poorer solvent, giving the dendrimer a more compact size.[10,11]

Other potential applications for dendritic macromolecules include their possible use as models for branched polysaccharides, such as amylopectin and arabinogalactan. Amylopectin is one of the main components of starch and is composed of α-(1→4) oligomers linked by α-(1→6) bonds to other α-(1→4) chains. It is highly branched, with molecular weights spanning the 10^7-10^9 range.[12] Even though much work has been done in the area of starch characterization, an appropriate structural model for amylopectin is still lacking. Maltodextrins are the products obtained when starch is subjected to enzymatic and/or hydrolytic degradation. They are nonsweet, nutritive saccharide polymers that consist of D-glucose units linked primarily by α-(1→4) bonds and containing a small but undetermined amount of α-(1→6) linkages. Maltodextrins have a dextrose equivalent (DE) of less than 20.[13] Branching in dextrins is unspecified but believed to be low.

As an interest exists in comparing dextrins to molecules with a known, ordered architecture, we have, as an initial step, contrasted their dilute solution behavior to that of Starburst dendrimers (Figure 1, top) and linear dextrans (α-(1→6)-linked polysaccharides, Figure 1, bottom) of equivalent molar mass. Size exclusion chromatography (SEC) applying the concept of universal calibration was used to determine molecular weight averages, distributions, intrinsic viscosities, and structural characteristics of the various natural and synthetic polymers examined. Molecular dynamics computer modeling using force fields yielded sizes of the dendrimers in solution at conditions similar to those employed

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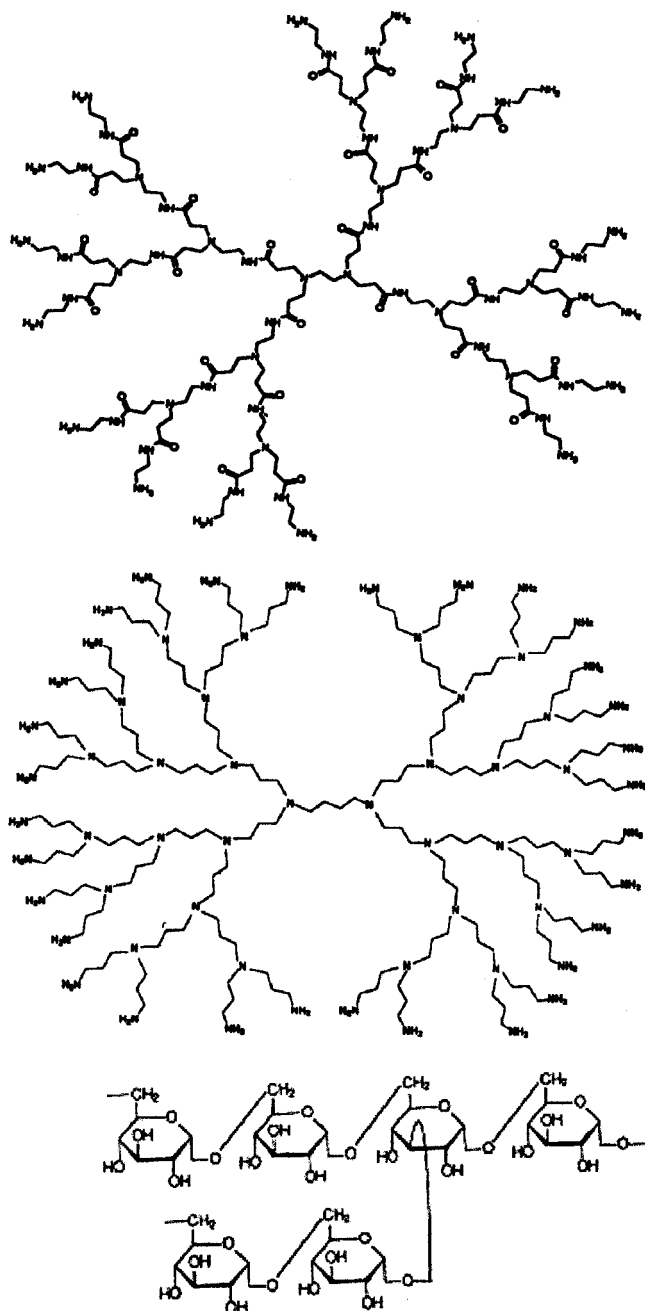(13) Krueger, W. J. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1995.

Figure 1. Structures of (top) generation 2 Starburst poly(amido amine) dendrimer (middle) generation 4 Astramol poly(propylene imine) dendrimer, and (bottom) Dextran.

in the chromatographic experiments. For Astramol dendrimers (Figure 1, middle), which showed limited solubility in the solvent/ mobile phase of choice, mass spectrometric analysis using a soft-ionization method was used to calculate molecular weight averages and polydispersities.

EXPERIMENTAL SECTION

Size Exclusion Chromatography. SEC experiments were carried out on a Waters 150 CV+ instrument (Waters Associates, Milford, MA) equipped with both differential refractive index and single-capillary viscometer detectors. The solvent/mobile phase was H_2O/0.02% NaN_3, at a flow rate of 1.0 mL/min. Pump, solvent, and detector compartments were maintained at 50 °C. Separation

occurred over a column bank consisting of three analytical columns preceded by a guard column, Shodex KB-G, KS-802, KS-803, and KS-804 (Phenomenex, Torrance, CA). Universal calibration was performed using a series of oligosaccharides (Sigma, St. Louis, MO) and pullulan standards (American Polymer Standards, Mentor, OH, and Polymer Laboratories, Amherst, MA). Molecular weight, intrinsic viscosity, and branching index calculations were performed using Millennium software (Waters, v. 2.15), applying Savitsky−Golay filtering using 17 data points.

Mass Spectrometry. Mass spectrometric experiments were carried out on either a TSQ 700 triple-quadrupole instrument (Finnigan MAT, San Jose, CA) or an LCQ quadrupole ion trap instrument (Finnigan MAT), both equipped with an electrospray ionization source. All spectra were acquired in the positive ion mode. On the TSQ, the capillary was maintained at 250 °C, the needle voltage was 4.5 kV, with pressures in the quadrupole and sampling cone regions measuring 1×10^{-6} Torr and 0.8 mTorr, respectively. Direct infusion was performed at a rate of 25 μL/ min. Solution concentrations were 120 and 100 ng/μL for Astramol 4 and 5, respectively, in 1:1:0.01 methanol/water/acetic acid.

ZoomScan analyses were conducted on the LCQ quadrupole ion trap instrument. The capillary was maintained at 220 °C and 4.5 V, the needle (spray) voltage was 4.5 kV, and the ion gauge and convection gauge pressures were 2.33×10^{-5} and 0.72 Torr, respectively. Nitrogen was used as a sheath gas, at a flow rate of 80 mL/min. The rate of direct infusion was 10 μL/min. Concentrations were the same as above.

Computer Modeling. All dendrimer building and molecular dynamics simulations are based on the consistent valence force field (CVFF) of the Insight II software (Molecular Simulations Inc., San Diego, CA). Each construction consisted of a dendrimer surrounded by a minimum 10-Å layer of water molecules. The latter were not constrained during the molecular dynamics run at 323.16 K (50 °C). Mean radii of gyration were calculated from molecule configurations created by a molecular dynamics run of 1-ps duration. Modeling was performed using a Silicon Graphics Inc. 150-MHz Indigo2 system.[14]

Materials. Starburst poly(amido amine) dendrimers are manufactured by Dendritech, Inc. (Midland, MI). Generation 3 Starburst was acquired from Aldrich (Wilwaukee, WI), generations 4 and 6 were from Polysciences, Inc. (Warrington, PA). Astramol poly-(propylene imine) dendrimers are manufactured by DSM (Geleen, The Netherlands) and were purchased from Aldrich. Dextrans 7.2, 11.7, and 50.8 were purchased from American Polymer Standards. Dextran 20 is from Pharmacia (Uppsala, Sweden). Maltodextrins are from Grain Processing Corp. (Muscatine, IA).

Starburst dendrimers are sold as 10% (generations 4 and 6) or 20% (generation 3) solutions in methanol. Methanol was evaporated with nitrogen bath gas, the dendrimers were dissolved in H_2O/NaN_3, and solutions were heated to 80 °C to remove any remaining methanol. As regards the nomenclature of the various dendrimer species, in Starburst dendrimers the core is assigned a generation number of 0, while in Astramol dendrimers it is assigned a generation number of 1. The most obvious distinction when viewing or drawing structures for these molecules becomes the number of terminal surface groups. Starburst 4, for example, will have 64 terminal surface groups, whereas Astramol 4 will have

(14) Insight/Discover, Version 96.0. San Diego: Molecular Simulations, 1996.

Table 1. Molecular Weight and Intrinsic Viscosity Data for Starburst Dendrimers and Dextrans

sample	M_w (supplied)	M_w (SEC)	M_n (SEC)	PD (SEC)	$[\eta]$ (dL/g) (SEC)	g' (SEC)
Starburst 3	6 909[a]	6 685	6 604	1.01	0.05	
Starburst 4	14 215[a]	13 936	13 484	1.03	0.04	
Starburst 6	58 000[a]	54 508	53 952	1.01	0.03	
Dextran 7.2	7 200	7 420	2 686	2.76	0.08	1.03
Dextran 11.7	11 700	11 604	6 400	1.81	0.11	1.03
Dextran 20	20 000	22 364	13 216	1.69	0.15	1.10
Dextran 50.8	50 800	50 317	28 768	1.75	0.25	0.98

[a] Supplied molecular weights for Starburst dendrimers are theoretical averages. See text, eqs. 1 and 2.

Table 2. Molecular Weight and Intrinsic Viscosity Data for Maltodextrins

sample	DE	M_n (from DE)[a]	M_w (SEC)	M_n (SEC)	PD (SEC)	$[\eta]$ (dL/g) (SEC)	g' (SEC)
Maltrin M040	5	3240	90 747	2779	32.7	0.11	1.30
Maltrin M100	10	1620	42 394	1974	21.5	0.06	0.89
Maltrin M180	18	900	25 172	957	26.3	0.05	1.40

[a] See text, eq 3.

32. Starburst 5 will have 128; Astramol 5 will have 64.[15,16] Recently introduced guidelines for the nomenclature of dendritic polymers should help eliminate confusion arising from these differences in nomenclature.[17]

RESULTS AND DISCUSSION

Dendrimers vs Linear Polysaccharides. The solution behavior of several generations of Starburst poly(amido amine) dendrimers (Figure 1, top), low-molecular-weight ($M_w < 60\,000$) dextrans (Figure 1, bottom), and maltodextrins was examined by size exclusion chromatography. While generally referred to as branched molecules, it has long been known that branching does not express itself markedly in dextrans until they reach a certain high-molecular-weight stage.[18] The exact placing of this high-molecular-weight limit is uncertain, with recent reports as high as 250 000 and higher.[19] It is generally believed that dextrans below this molecular weight may be considered linear, as branching in these polysaccharides is both short-chained and the mass of the chain arms constitutes a very small percentage of the total molar mass of the molecule.

Performing SEC with dual detection (DRI and viscometry) permitted application of the concept of universal calibration, whereby the hydrodynamic volume of the molecule (defined as the product of the intrinsic viscosity and the molecular weight) is related semilogarithmically to the elution time (or volume).[20] Universal calibration allows the determination of absolute molecular weight averages without the need for standards with chemical composition and/or conformation identical to those of the analyte. This approach also yields information in the form of molecular weight polydispersities and distributions and intrinsic viscosities. As may be seen in Table 1, M_w values obtained by this method

closely approximate those supplied by the various manufacturers. It is also evident that the polydispersities (PD = M_w/M_n) of the synthetic polymers are narrower than those of the dextrans or, more markedly, than the polydispersities of the maltodextrins (Table 2). For Starbursts and Astramols, supplied M_w values are theoretical average molecular weights (M) calculated from eq 1,[21]

$$M = M_c + N_c\{M_{RU}[(N_b{}^{G+1} - 1)/(N_b - 1)] + M_t N_b{}^{G+1}\} \tag{1}$$

where M_c, M_{RU}, and M_t are the molecular weight of the initiator core, repeat units, and terminal units, respectively, N_c and N_b are the initiator core multiplicity and branch-juncture multiplicity, respectively, G is the dendrimer generation, and the degree of polymerization of the dendrimer (number of dendrimer repeat units, N_{RU}) may be calculated from eq 2

$$N_{RU} = N_c[(N_b{}^{G+1} - 1)/(N_b - 1)] \tag{2}$$

Weight-average molecular weights for the dendrimers determined by SEC with universal calibration using oligo- and polysaccharide narrow standards were slightly, albeit consistently lower than the theoretical averages. Other authors have noted that SEC with narrow polystyrene (PS) standards underestimates the molecular weight of dendrimers and have attributed this behavior to the dendrimers becoming denser and more compact with increasing molecular weight.[22,23] At least one report exists in which molecular weights based on SEC with PS standards were higher than the calculated values.[24]

Starburst generations 3 and 4 (hereafter referred to as Starburst 3 and Starburst 4, respectively) may be compared to

(15) Dendritech technical bulletin, 1995.
(16) DSM supplement 08-96/1.000.
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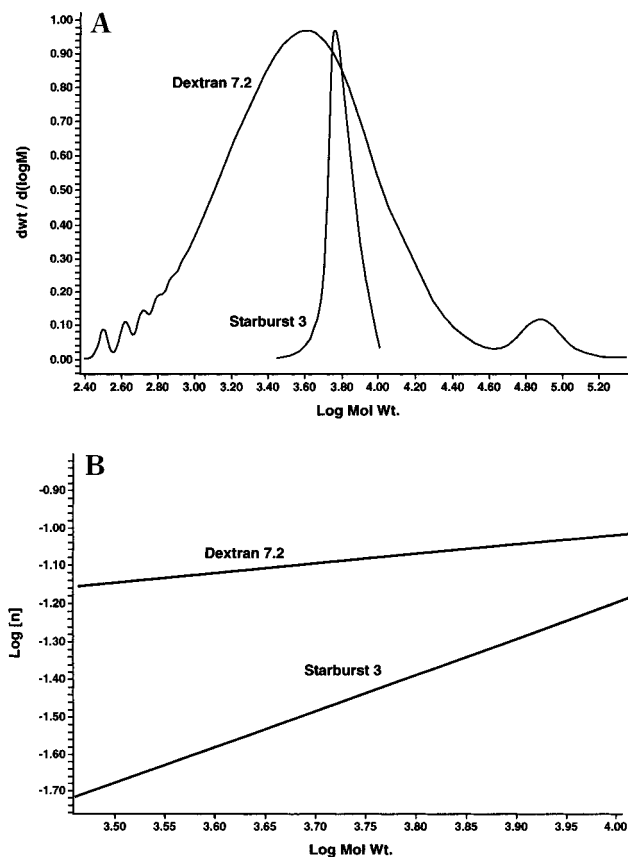
Figure 2. (A) MWD of Starburst 3 vs Dextran 7.2. (B) Mark−Houwink plot of Starburst 3 vs Dextran 7.2.

linear dextrans of equivalent molar mass, to note the differences in solution behavior of these structurally diverse polymers. For example, even though Starburst 3 and Dextran 7.2 have similar M_w's (∼7000), the former possesses a narrower molecular weight distribution (MWD) than does the latter (Figure 2A). The intrinsic viscosity, $[\eta]$, of the dendrimer is seen to be lower than that of the dextran, both in its average value (Table 1) and throughout the range of its MWD (Figure 2B). This is due to the high degree of branching present in the dendrimer, which affords this molecule a tighter coil density and, thus, a solution with lower intrinsic viscosity than that of the linear polysaccharide. Consistent with these observations are those obtained when contrasting the next generation of dendrimer, Starburst 4, with Dextran 11.7 (Figure 3A,B), both of which also have similar M_w's.

M_n of Maltodextrins. Maltodextrins are classified and sold according to their DE. DE is an indication of the reducing sugar content of a sweetener, calculated as dextrose and expressed as a percentage of the total dry substance. It is indicative of the percentage of dextrose (glucose) units, which are at reducing ends of the molecule. For example, a DE of 5 means that 5% of glucose units are reducing ends of the molecule. As such, the number-average molecular weight (M_n) may be calculated from the DE by eq 3, where 162 corresponds to the molecular weight of the

$$M_n = 162(100/DE) \tag{3}$$

anhydroglucose repeat unit. Averages calculated by this method correspond closely to those obtained using SEC with universal
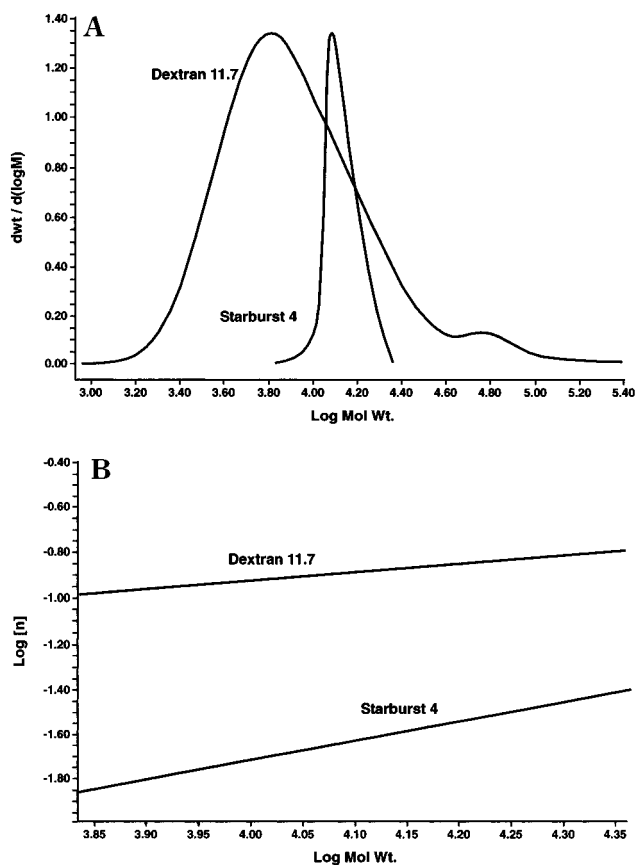

Figure 3. (A) MWD of Starburst 4 vs Dextran 11.7. (B) Mark−Houwink plot of Starburst 4 vs Dextran 11.7.

calibration (Table 2). For Maltrin M040, calculated and chromato-graphically determined values agree well with the M_n of 3300 obtained using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.[13]

Branching Index. Branching information derived from SEC data for polymers is normally obtained according to the theory developed by Zimm and Stockmayer.[25] In this approach, a linear standard is analyzed and a ratio of mean square radii is derived from comparing the intrinsic viscosities, the root-mean-square radii of gyration, or a molecular weight average of a branched sample to the values for the standard. From this, branching number (number of branches per molecule), branching frequency (number of branches per molecule per repeat unit of molecular weight), etc., are calculated. This approach has been used to describe the differences in solution behavior of linear and branched polysac-charides analyzed by SEC.[19]

The linearity of polymers as calculated by the SEC Millennium software used in these studies is expressed as a branching index, g', given by eq 4, which compares the deviation, caused by

$$g' = [\eta]_{branched}/[\eta]_{linear} \tag{4}$$

branching, of the Mark−Houwink intrinsic viscosity vs molecular weight relationship extrapolated from the lower molecular weight, linear portion of the molecule.[26] No linear standard is used as a

(25) Zimm, B. H.; Stockmayer, W. H. *J. Chem. Phys.* **1949**, *17*, 1301−1314.
(26) Waters supplement 063021TP, Rev0, 1994.

basis for comparison. g' should have a value of 1.00 for linear molecules and decrease with an increase in branching. Branching index values for the dextrans studied are near unity (Table 1). This confirms the assumption that the lower molecular weight dextrans may be considered linear molecules. The values for the maltodextrins span the range from 0.89 to 1.40. It is, of course, physically impossible to have $g' > 1$. The inflated values for the Maltrins are likely artifacts of the low signal-to-noise ratio at the low-molecular-weight end of the MWDs due to the multimodality of the distributions. They may also be due to a small amount of residual branching along the backbone of the polysaccharides, rendering the lower portion of the MWD less than ideal for linear extrapolation. A method such as the one described in the previous paragraph involving comparison to a linear standard, should such a standard exist for the dextrins, would be expected to yield more accurate results. Branching index calculations carried out by the Millennium software method, moreover, have no physical meaning when applied to dendritically branched systems, as no linear portion exists in these molecules from which to extrapolate the $[\eta]$ vs M curve. Consequently, no g' values are reported for these systems. A similar limitation may be expected to exist in hyperbranched systems. As with the maltodextrins, one may assume that a method in which the dendrimers are compared to a linear standard would be better suited to estimating the branching of these molecules. Recent work on the synthesis of exact linear analogues of polyether dendrimers is a step in this direction.[27]

Mark−Houwink Relationships. In general, the intrinsic viscosity of polymers tends to increase with increasing molecular weight (M), which accompanies an increase in size of the macromolecule. Exceptions to this are hyperbranched polymers, in which the Mark−Houwink double-logarithmic $[\eta]$ vs M curve passes through a minimum in the low-molecular-weight region before steadily increasing.[28] For the dendrimers studied in solution in these experiments, it is evident that as M increases, $[\eta]$ decreases (Table 1). This corresponds to the molecules growing faster in density than in radial growth. Fréchet has pointed out the special situation of this class of polymers, in which their volume increases cubically whereas their mass increases exponentially.[1] This is a unique characteristic of dendritically branched systems. The resultant inversion in the Mark−Houwink relationship, previously reported to occur in dendrimers around generation 3 or 4,[1,29] is postulated to result from a morphological transformation from a planar, disklike structure to a spherical architecture. Thus, the $[\eta]-M$ curve will proceed through a maximum around generation 3 or 4 before taking on a negative slope. From the data in Table 1, it may be concluded that this change in molecular shape has already occurred by generation 3 in the Starbursts under the present solution conditions. For the dextrans and maltodextrins, intrinsic viscosity follows the normal pattern of increase with increasing molecular weight (Tables 1 and 2).

When comparing Maltrin M100 to Starburst 6 and to Dextran 50.8, all of which have similar M_w's, the large polydispersity of
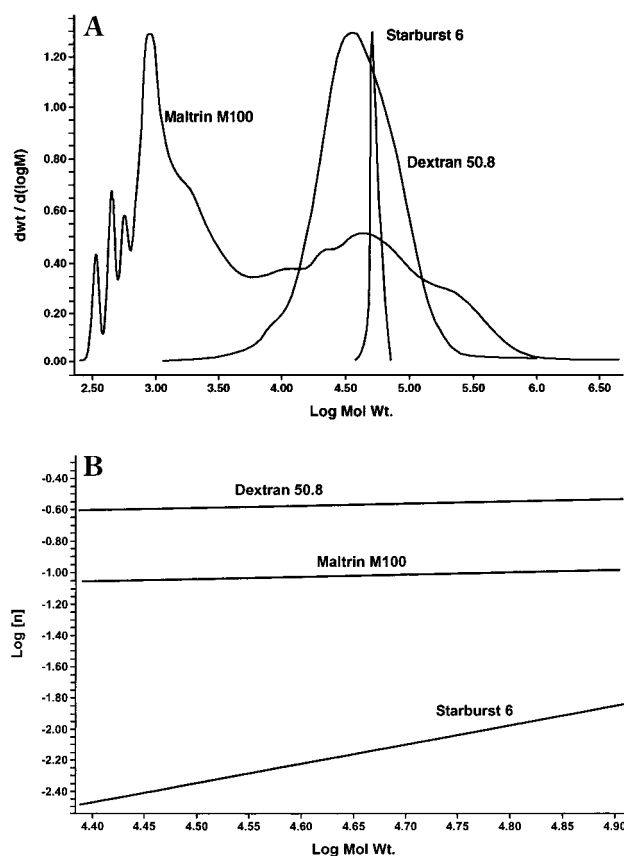
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Figure 4. (A) MWD of Starburst 6 vs Dextran 50.8 vs Maltrin M100. (B) Mark−Houwink plot of Starburst 6 vs Dextran 50.8 vs Maltrin M100.

the maltodextrin is immediately evident (Tables 1 and 2). This is a reflection of its broad, multimodal MWD (Figure 4A), as opposed to the narrower, monomodal distributions of the dextran and the Starburst. As evidenced by Figure 4B, the intrinsic viscosity of the solutions decreases in the order $[\eta]_{dextran} > [\eta]_{maltodextrin} > [\eta]_{dendrimer}$. The tighter coil density caused by branching in the dendrimer manifests itself in the lower intrinsic viscosity (Tables 1 and 2, Figure 4B) as compared to both polysaccharides. The exponent a in the Mark−Houwink equation (5) for these polymers

$$[\eta] = KM^a \tag{5}$$

is 0.6 for both dextrans and maltodextrins, in the range (0.5−0.8) of a flexible linear coil.[30] The exponent for the dendrimers is −0.2 for the generations studied (located in the "inverted" region of the Mark−Houwink plot). This value for the Starbursts is comparable to the a value of −0.2 for convergent growth polyether dendrimers, generations 3−6, studied by Mourey et al.[29] (both Starburst and Astramol dendrimers are made by divergent synthetic methods). The lower intrinsic viscosity of the dendrimer is readily explained by the high degree of branching present in its structure. To understand the differences in intrinsic viscosity between the dextrans and the maltodextrins, we examine the values of the coefficient K of the Mark−Houwink equation. Several factors can influence the value of this constant: solvent, bond

(30) Vollmert, B. *Polymer Chemistry*; Springer-Verlag: New York, 1973; p 520.

dimensions, and freedom of rotation about bonds, among others.[31] Previous experiments have compared dextran (comprising α-(1→6) linkages), amylose (comprising of α-(1→4) linkages), and pullulan (comprising of two α-(1→4) linkages per α-(1→6) linkage).[32] All three polysaccharides had equal values of a (~0.7), but K varied in the order $K_{dextran}$ (4.9 × 10^{-4} dL/g) > $K_{pullulan}$ (2.5 × 10^{-4} dL/g) > $K_{amylose}$ (1.3 × 10^{-4} dL/g). The average values for K in our experiments are 4.6 × 10^{-4} dL/g for the dextrans and 8.2 × 10^{-5} dL/g for the maltodextrins, the latter being composed of both α-(1→4) and α-(1→6) linkages. Our results agree with the observation that α-(1→4) polysaccharides have lower K values than α-(1→6) polysaccharides under equivalent solution conditions. The lower K value for maltodextrin compared to amylose suggests the former has a more highly branched structure than the latter, although further data are necessary to confirm this result.

Size of Dendrimers in Solution. Solution radii of polymers may be derived from SEC data by a variety of approaches. Using a multiangle (or variable-angle) light-scattering photometer as a detector, mean-square radii of gyration (R_g) can be evaluated from Debye, Zimm, Berry, Guinier, or Kratky plots, depending upon the conformation of the molecules in solution.[33] When a viscometer is used, hydrodynamic radii can be determined by the Flory−Fox method,[34] by the Ptitsyn−Eizner method,[35] or by a combination of these.[36] An alternative approach is the Hester−Mitchell method.[37] In this approach, Guth's modification of Einstein's viscosity relationship[38] is used to determine the hydrodynamic radius (R_η) via eqs 6 and 7.

$$R_\eta = d/2 \qquad (6)$$

$$d = [240/(\pi N_A)]^{1/2}(M[\eta])^{1/2} \qquad (7)$$

We have applied the Hester−Mitchell approach to SEC data generated in our laboratory in order to make comparison with reported Starburst radii more meaningful, as this is the method used by the manufacturers to determine molecular size, in addition to being a favorable method for molecules occupying a spherical volume in solution.[21] As may be seen in Table 3, R_η values for Starburst generations 3, 4, and 6 agree closely with those provided.

Radii of Astramol poly(propylene imine) dendrimers (Figure 1, middle) were determined by the manufacturer using small-angle neutron scattering (SANS).[16] Astramol dendrimers, however, displayed limited solubility in the chromatographic solvent/mobile phase used in the present experiments. Consequently, we were unable to calculate hydrodynamic radii for these molecules. To calculate sizes for both Astramol 4 and 5 and Starburst 3 and 4 dendrimers, we used molecular dynamics with a CVFF force

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Table 3. Solution Radii of Astramol and Starburst Dendrimers by SEC and Computer Modeling

dendrimer	R_g (Å) (supplied)	R_η (Å) (calcd)[a]	R_g (Å) (CVFF)[b]	ρ
Astramol 4	11.6[c]		10.8	
Astramol 5	12.9[c]		13.9	
Starburst 3	18.0[d]	17.4	18.9	1.09
Starburst 4	22.5[d]	20.6	22.4	1.09
Starburst 6	33.5[d]	29.5		

[a] From SEC M_w and [η] data. See text, eqs 6 and 7. [b] From computer modeling using CVFF force field. See text for details. [c] R_g determined using SANS (ref 16). [d] R_η determined using SEC in citrate buffer (refs 15 and 21).

field.[14] Construction of a generation 6 Starburst dendrimer for modeling purposes proved too logistically difficult to accomplish, as this molecule is composed of over 9100 atoms. Radii calculated by this method correspond to the root-mean-square distances of the atoms in the dendrimer from the molecule's center of mass, as defined by eq 8, where r_i are the positions of the ($n + 1$) atoms

$$R_g^2 = [1/(n+1)][\sum_i(r_i - R_{cm})^2] \qquad (8)$$

making up the backbone of a chain containing n bonds in its backbone and R_{cm} is the location of the chain's center of mass. Molecules were surrounded with a 10-Å layer of water, and the temperature was maintained at 50 °C to mimic the conditions of SEC analysis (without NaN$_3$). For the Astramols, radii of gyration obtained by this method correspond closely with results from SANS (Table 3). For the Starbursts, R_g values by computer modeling were similar to R_η values obtained both in our laboratory using SEC and to those provided by the manufacturer (Table 3).[15]

As mentioned earlier, SEC experiments with Starburst dendrimers showed they possess narrow MWDs and, consequently, small polydispersities (the small polydispersities of Astramols are discussed in the following section). These facts should reflect in a uniform distribution of sizes. Using computer modeling we have calculated the probability distribution of the radii of gyration, $P(s)$, for Starburst and Astramol dendrimers. For the fourth generation of each of these (Figure 5A,C), $P(s)$ plots (Figure 5B,D) allow visualization of the extremely small deviations in molecular size. A longer (9.5-ps) dynamics simulation of Starburst 3 appears to indicate that said deviations are due to a slight shrinking of the dendrimers over the course of the dynamics run.

It is instructive to examine the ratio of the geometric-to-hydrodynamic radii ($\rho = R_g/R_\eta$) for the Starbursts. The value of 1.09 for both Starburst 3 and 4 is intermediate between the values for a homogeneous sphere (0.78) and a θ-random coil (1.50).[39] Even though ρ-values have not been derived for dendritically branched molecules specifically, the data for the Starbursts fit approximately in the range of θ-stars with $f > 1$ (1.08) and $f = 4$ (1.33), in accordance with the trifunctionality of these polymers.

For a β-limiting dextrin, Burchard et al. calculated a value for ρ of 0.89,[40] also intermediate between a homogeneous sphere and

(39) Burchard, W.; Richtering, W. *Prog. Colloid., Polym. Sci.* **1989**, *90*, 151−163.
(40) Burchard, W.; Schmidt, M.; Stockmayer, W. H. *Macromol.* **1980**, *13*, 1265−1272.
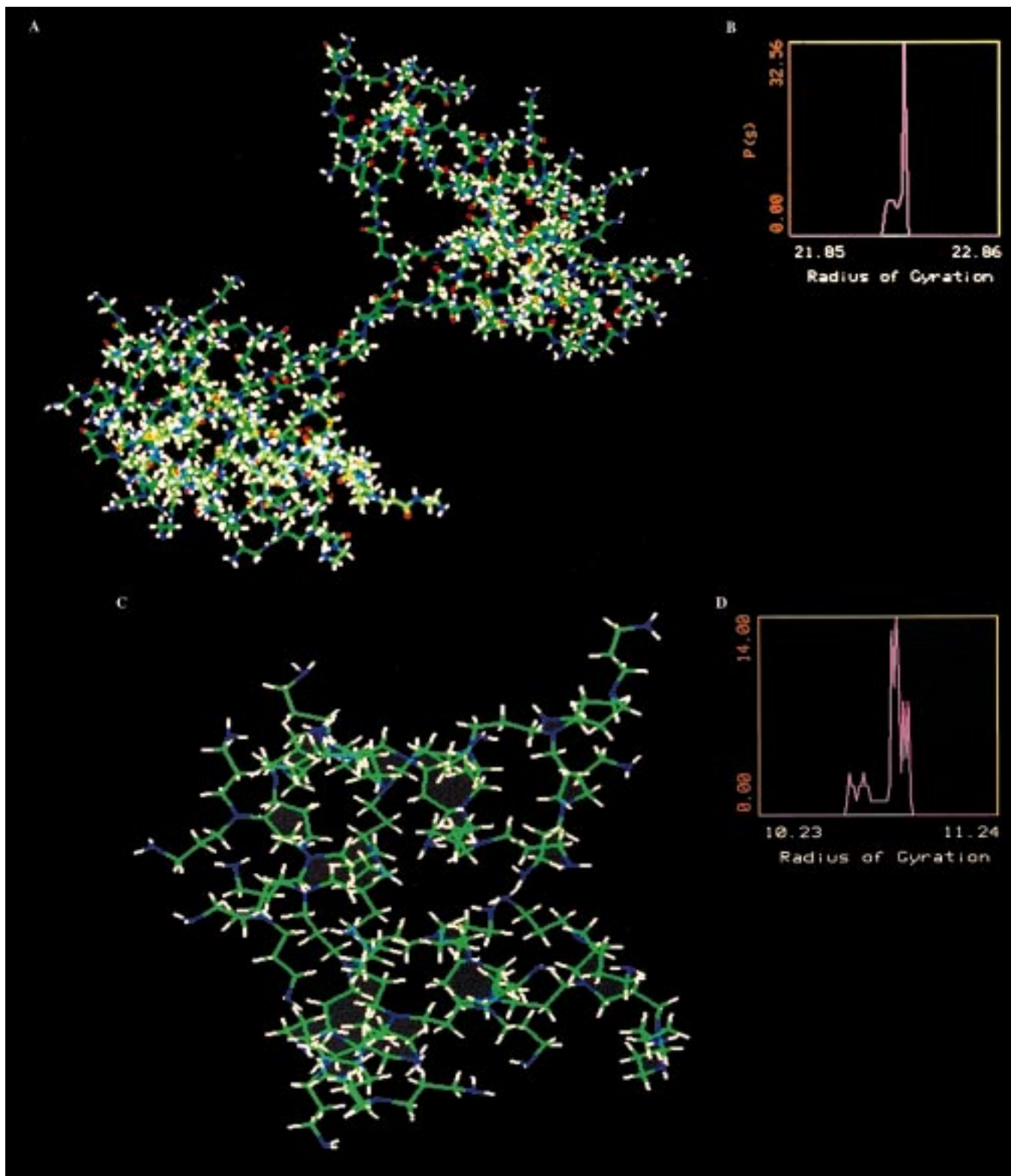
Figure 5. CVFF molecular dynamics model of (A) Starburst 4 and (C) Astramol 4. Probability distribution of R_g of (B) Starburst 4 and (D) Astramol 4.

a θ-random coil, but closer to the former. Such a dextrin is manufactured by the action of the exoenzyme β-amylase on amylopectin, which tends to degrade only the outer chains of the branched polysaccharide, leading to a more spherical structure in solution. The dextrins used in this study were manufactured by a combination of acid and enzyme degradation, although specific information about the identity of the enzymes involved is not available from the manufacturer. Based on the values of the branching indices (0.89−1.40) and the Mark−Houwink exponent a (0.6) of the Maltrins, a more thorough debranching appears to

have occurred in these polysaccharides than in the β-limiting dextrin studied by Burchard's group, as the Maltrins appear to behave more as flexible linear coils than as branched systems.

Mass Spectrometry of Astramols. Reference has been made to the difficulty in obtaining solutions of Astramol dendrimers (Figure 1, middle) for SEC analysis under the present conditions. While the initial goal was to compare these molecules to the other dendrimers and polysaccharides, the desire to characterize the Astramols, nonetheless, was motivated both by scientific curiosity and in order to gather data of potential use in future studies using a common solvent. This has led to other analytical methods for obtaining molecular weight averages and polydispersities. While mass spectrometry is not normally thought of as a technique that can provide such information, the soft-ionization spray methods currently available are ideal for such work (as is matrix-assisted laser desorption/ionization. The low energy transfer to the molecule inherent to these types of ionization is not conducive to fragmentation, thus allowing for visualization of an intact molecular ion.[41,42] Moreover, the multiple-charging mechanism by which these methods proceed provides one or several envelopes of peaks, all of which correspond to molecular ions at different charge states and, thus, permits calculation of molecular weight averages and polydispersities.[43,44]

Astramol generations 4 and 5 were examined using electrospray ionization mass spectrometry (ESI-MS) (Figure 6A), on a linear triple-quadrupole instrument, used in single-quadrupole mode. Following analysis by ESI-MS, molecular weight averages (M_w and M_n) were calculated from the Meyerhoff equation (9),[45]

$$M_{w,n} = [\sum(N_I M_i^{x+1})]/[\sum(N_I M_i^x)] \qquad (9)$$

where N_i is the mass spectrometrically determined peak intensity (in arbitrary units) of each multiply charged peak in the m/z envelope, M_i is the molecular weight of said peak (after compensating for both the charge state and the mass of the charge-carrier adducts), and $x = 0$ for M_n and 1 for M_w.

It may be observed that polydispersity values for both generations of Astramol dendrimers examined by this technique are essentially unity (Table 4). The extremely narrow polydispersities obtained by ESI-MS attest both to the completeness of the synthetic route by which the dendrimers were prepared and to the soft-ionization capability of this mass spectrometry technique, which greatly reduces fragmentation of the dendrimers.

The charge state of a molecule in ESI-MS and, thus, its molecular weight, may be determined from two adjacent peaks in the mass-to-charge envelope, as long as the identity of the charge carrier is known (usually, though not necessarily, this will
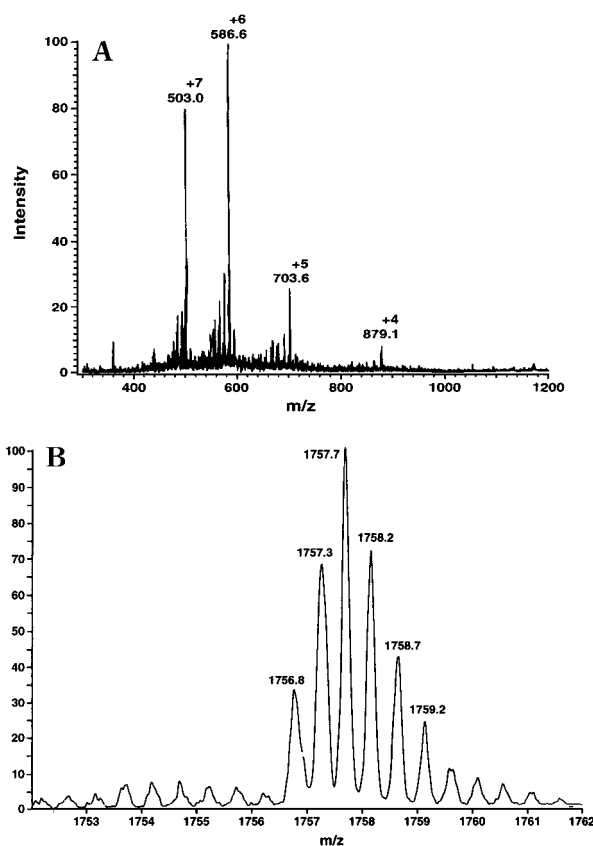

Figure 6. (A) ESI-MS spectrum of Astramol 4. (B) ZoomScan spectrum of 2^+ charge state of Astramol 4.

Table 4. Molecular Weight Averages and Polydispersities of Astramols by Electrospray Ionization Mass Spectrometry

sample	M_w (supplied)[a]	M_w (MS)[b]	M_n (MS)	PD
Astramol 4	3514	3513.0	3513.0	1.0000
Astramol 5	7168	7168.1	7168.1	1.0000

[a] Supplied molecular weights are theoretical averages. See text, eqs 1 and 2. [b] MS refers to values determined using ESI-MS. See text, eq 9.

be a proton, as in the present case).[46] Averaging over a higher number of peaks will increase the precision of the determinations. A method now exists, using quadrupole ion trap (QIT) technology, to unambiguously determine the charge state of a molecular ion. Previously, high-resolution mass analysis had been conducted using Fourier transform ion cyclotron resonance and Fourier transform tandem mass spectrometers.[47,48] When operating in ZoomScan mode, the LCQ QIT mass analyzer scans a narrow mass range at high resolution to determine the difference in mass-to-charge ratio of two adjacent isotope peaks ($\Delta m/z$).[49] The

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(49) Bier, M. E.; Schwartz, J. C. In *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation, and Applications*; Cole, R. B., Ed.; John Wiley & Sons: New York; 1997; p 258.

difference between adjacent peaks corresponds to $1/$(charge state). From this the charge state is assigned and, hence, the mass. In Figure 6B, the charge state of the Astramol 4 peak at m/z 1758 is examined. The peak multiplicity corresponds to the isotopic distribution of the molecule. In the present case, the difference of 0.5 indicates a 2^+ charge state. The value of this type of analysis lies not only in confirming the charge states calculated from spectra obtained with linear quadrupole (or other type of mass analyzer) instruments but also in determining charge states and masses in cases where the number of multiply charged peaks in said spectra is small, causing precision to be low, or when the identity of the charge carrier is unknown.

CONCLUSIONS

The analysis of Starburst dendrimers, dextrans, and malto-dextrins by means of size exclusion chromatography with universal calibration allowed comparison of molecular weight averages and distributions of these compounds. When contrasting species of similar M_w, the dendrimers were observed to possess a narrower molecular weight distribution, which reflected in polydispersity values closer to unity. The extremely high degree of branching in the dendrimers caused these to have a tighter coil density than the linear polysaccharides to which they were being compared. This smaller size in solution resulted in a lower intrinsic viscosity for solutions of the dendrimers than for those of the dextrans or maltodextrins throughout the range of their MWDs. As the Starbursts do not appear to be adequate models for maltodextrins, future work in this area is likely to concentrate on different degradation products of starch as well as on higher generation dendrimers. Currently commercially available dendrimers have molecular weights as high as 1 million (generation 10 Starbursts).

SEC incorporating a viscosity detector also permitted calculation of hydrodynamic radii of the Starburst dendrimers. Molecular sizes determined by this method agreed closely with those arrived at by the manufacturers through various analytical techniques. Additionally, application of computer modeling to the calculation of root-mean-square radii of gyration allowed comparison between R_g and R_η values for the Starbursts, as well as yielding R_g data for difficult-to-dissolve Astramols. The narrow probability distributions of the R_g's for both sets of dendrimers attest to the uniformity in size of these synthetically constructed polymers. When the results from SEC are combined with those from computer modeling by comparing the ratios of geometric to hydrodynamic radii for the trifunctional Starbursts to the ratios derived for other molecular geometries, the dendrimers appear to resemble θ-stars with functionalities between 1 and 4. Debranching during the manufacture of the dextrins studied here appears to have been more thorough than in that of β-limiting dextrins previously examined, based on contrasting structural data obtained via SEC to that obtained by other groups using a combination of scattering methodologies.

Application of electrospray ionization mass spectrometry methods to the analysis of Astramol dendrimers resulted in the determination of molecular weight averages and polydispersities for these molecules. While PD values obtained for Starburst dendrimers using SEC are certainly narrow ($\sim$1.01), the values obtained for Astramols using ESI-MS are essentially unity (1.0000). This is likely due to both the shear forces and band broadening experienced by the Starbursts in their passage through the SEC columns and not to a less-optimized synthetic route in the manufacture of these polymers. Use of quadrupole ion trap methodology also permitted unambiguous assignment of charge states to peaks in the mass-to-charge envelope of the Astramols, thereby allowing for a more straightforward calculation of mass.